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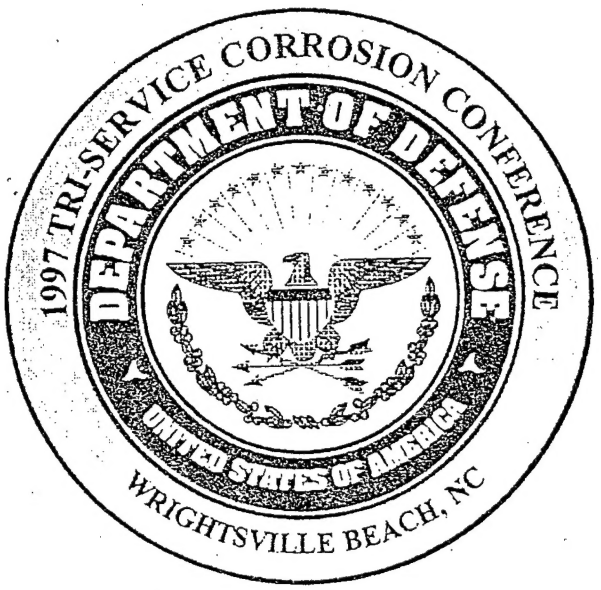

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Inhibition of Microbiologically Influenced Corrosion

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ABSTRACT

Two areas of work designed to provide a better understanding on the nature of microbial colonization of surfaces and microbiologically influenced corrosion are discussed. The first approach examined a mechanical sensing model to describe microbial colonization. Results obtained after the exposure of a group polydimethylsiloxane polymers to an estuary environment provide support for this model. The second approach sought to determine if there is a relationship between microbial colonization of certain metallic substrates and corrosion. Exposure of zirconium, chromium, niobium, tantalum, molybdenum, tungsten and 4340 steel to Postgate medium with 2.5% NaCl and sulfate reducing bacteria (SRB) resulted in biofilms being formed on all samples. In the case of the zirconium, chromium, niobium, and tantalum samples, the weight loss after two years of exposure was negligible.

INTRODUCTION

Bacteria and other microorganisms attach and colonize the surface of metals that are exposed to aquatic environments and form biofilms [1-6]. The physical presence and metabolic processes of the microorganisms in the biofilm produce chemical changes at the biofilm/metal interface. In particular, the biofilm alters the dissolved oxygen concentration, pH, and the concentration of organic and inorganic species at the metal surface. These changes, in turn, alter the electrochemical characteristics of the metal and thereby influence its corrosion behavior. This process is referred to as microbiologically influenced corrosion (MIC). Biofilm formation resulting in MIC has been recognized by the engineering community as a significant factor in reducing the useful lifetime of equipment [2-5].

It is well known that microorganisms can change their surface characteristics in response to changes in the environment [6-9]. For example, it has been demonstrated that physiological differences exist between the sessile and planktonic cells of the same

bacterium[6-9]. The characteristic that is emphasized here is the exceptional ability of microorganisms to respond to environmental stimuli. The exact nature of the stimulus/stimuli that trigger phenotypic changes in microorganisms allowing them to become irreversibly adsorbed onto metal surfaces is not totally clear and could be chemical and/or physical in nature. This paper summarizes the research, to date, on two areas of work that were designed to provide a better understanding of colonization on surfaces and provide means to prevent the colonization which can lead to biofouling and/or MIC. The first approach discussed is based on an assumption that the major prerequisite for microbial colonization is a solid surface. The second seeks to determine if there is a relationship between microbial colonization, the type of metallic substrate, and corrosion. This paper reviews results presented elsewhere [10,11].

Dimethylsilcones and the Mechanical Sensing Model

Microbes do not colonize all surfaces equally, but exhibit preferences among immersed materials. There are two classes of materials which seem to be much more effective than others in resisting colonization: the dimethylsilicones (DMS) and the polyethylene oxide (PEO) linear polymers. The monomeric units of which these polymers are composed are quite different in their properties. DMS is a hydrophobic material with exposed methyl groups, while the PEO chain is a hydrophilic material due to the exposed ether oxygen atoms present in every monomeric unit, and which are hydrogen bonding sites available for interaction with water. The difference implies that if there is a common mode for preventing attachment by these materials, it must lie in the properties of the polymer as a whole. There is a group of properties of these polymers which is shared: it is their chain mobility and consequent high configurational entropy, and low resistance to deformation of the polymer chains at the material surface.

Belas et. al. [12] have shown that certain marine vibrio bacteria, which can be cultured in either a liquid medium or on a solid gel surface, change form depending on the situation. This change is quite dramatic with the cells from liquid medium having flagellae at one end with which they swim; while cells from a solid medium have lateral flagellae which grow out from a large number of sites along the lateral surface of the cells. The change between these forms is controlled by the activation of a particular set of genes when the organism senses that it is on or close to a surface. The only stimulus which has been found to induce this change (other than the presence of a surface) is a great increase in the viscosity of the medium in the presence of low iron concentrations[13]. Thus, it appears that a mechanical sensing mechanism is involved. There are many mechanical sensory cells known in biology and they seem to operate in the same way. That is, a deformation of the cell membrane causes a change in the ionic currents across it, which are controlled by membrane pore structures. In addition to this mechanism having been found in sensory cells whose function is to sense mechanical stimuli, recent reports indicate that this phenomenon is more general. Cells which normally function in a coherent tissue require a surface on which to grow if they are to function properly. These cells are called "anchorage dependent". Their functioning seems to depend upon their approaching a surface suitable for them (upon which they lose their rounded liquid-medium shape) and spread out to a much flatter cell with an increased number of attachment points to the immersed surface. Surfaces upon which strong attachment points do not seem to form do not lead to the change in cell shape which is correlated with their normal functioning. Leonid Margolis and co-workers [14-16] reported that attachment of animal tissue cells to such surfaces causes a change in the ratio of cellular ionic fluxes, so that the internal pH of cells changes when they accomplish proper attachment.

Thus, a signal dependent on the stressing of the cell membrane, thereby affecting membrane ion flux, appears to be required for the transformation of anchorage-dependent cells into their characteristic form for normal function on a surface or in a tissue. A surface which has very little tendency to induce such changes in cells is poly-DMS.

Metals

When a bare metal is exposed to a saltwater environment, it interacts electrochemically with the environment. The result is that metal ions can be present in the near surface region at concentrations greater than in the bulk aqueous environment. It has been observed that many microorganisms are able to sense and move toward nutrients and other attractants while moving away from harmful substances or other repellents. Movement toward chemical attractants and away from repellents is known as chemotaxis. Microorganisms can respond to very low levels of attractants on the order of 10^{-8} molar with the magnitude of the response increasing with attractant in this low concentration range[17]. Thus, it might be expected that metal ions produced by the anodic reaction and which microorganisms need as metabolites could attract microorganisms to the metal surface and/or stimulate or trigger attachment of the microorganisms leading to biofilm formation. MIC has been documented for metals exposed to sea water, fresh water, process chemicals, food stuffs, soils, aircraft fuels, human plasma, and sewage. The surface to which microbes attach plays a major role in biofilm processes during biofilm accumulation[4]. SRB may initiate anodic processes on metal surfaces by direct contact in a biofilm or by-products of bacterial metabolism (i.e. hydrogen sulfide) can contribute to corrosion processes. The purpose of this work is to compare biofilm development on pure metallic substrates using environmental scanning electron microscopy (ESEM) to characterize the topography of the wet biofilm. Weight loss measurements were done to evaluate whether metallic corrosion had occurred after a 2 year exposure to marine, SRB consortia.

EXPERIMENTAL

Dimethylsilcones

Two candidate silicone formulations (PEG-015 and PEG-060) were purchased from United Technologies. PEG-015 and PEG-060 are polydimethylsiloxane polymers with the 15 and 60 designations referring to chain length before catalytic cross-linking (polymerization). Films of varying rigidity were prepared by mixing the two liquid components, a prepolymer and a catalyst, in varying ratios (by weight). The films of varying rigidity were used to examine the significance of the mechanical sensing mechanism in regard to microbial attachment

Samples were cut from a 1 inch in diameter rod of type 4340 steel in segments that were 1/2 inch in thickness and were polished to a 600 grit finish. The samples were then coated with either the PEG-015 or PEG-060 using a mold technique. A mold was made and placed around the polished face and the PEG compounds were poured into the mold and allowed to dry. The thickness of the coatings ranged from 230 to 850 μm. Film thickness was measured by light section microscopy, making a refractive index correction by comparison with a sample of a silicone film cast on a microscope slide thick enough to be measured with a micrometer.

Silicon coated 4340 steel samples were also prepared by dip coating. Rectangular coupons (0.75" by 2") with a 600 grit surface finish were suspended in the liquid resin and withdrawn vertically using an electric motor at a rate of 1 mm per minute. Coupons were hung to cure in an air-conditioned room at 25° until the residual liquid in the mixing cup became rigid, usually 24 hours. Coating thicknesses ranged from 22 to 60 μm. In initial tests, corrosion occurred at the edges of the dipped sample due to thinning of the coatings at the sharp edges. After dipping and curing, samples were then held in contact with a small amount of additional liquid resin in a trough to cover the edges and these additional portions of polymer were allowed to cure. This process provided a very thick (1-2 mm) covering of polymer at the edges of the samples. Samples were then examined in abiotic electrochemical tests. Also, silicone samples were immersed in the estuary at the Smithsonian Laboratory in Edgewater, Maryland for six weeks.

Abiotic, electrochemical measurements were made in quiescent 0.6M NaCl solution using a three-electrode set-up in a conventional corrosion cell. Samples were immersed in the solution for 24 hours prior to polarization to establish a steady state open circuit potential. Anodic and cathodic polarization curves for treated and untreated 4340 steel samples were determined potentiostatically by stepping the potential in 25 or 50 mV increments from the open circuit potential in the anodic or cathodic direction and allowing the current to reach a steady state value. Usually 15 to 20 minutes were required at each potential. The polarization curves were used to detect defects in the coating. The *ac* impedance measurements were made during a 10 day period in quiescent 0.6M NaCl solution using a conventional corrosion cell. Potentials are reported relative to the saturated calomel electrode (sce).

Exposure of Pure Metals to Sulfate Reducing Bacteria Consortia

MIC has been documented for metals exposed to sea water, fresh water, process chemicals, food stuffs, soils, aircraft fuels, human plasma, and sewage. The surface to which microbes attach plays a major role in biofilm processes during biofilm accumulation[4]. SRB may initiate anodic processes on metal surfaces by direct contact in a biofilm or by-products of bacterial metabolism (i.e. hydrogen sulfide) can contribute to corrosion processes. Multiple samples (1/2" square) of tantalum, tungsten, chromium, niobium, molybdenum, zirconium, and 4340 steel were exposed to either a hydrogenase positive (49Z) or a hydrogenase negative (CG-59) SRB culture, as well as, Postgate medium (Control; no bacteria added). The isolation, maintenance, and characterization of sulfate reducing bacteria (SRB) communities have been described elsewhere [18-21]. The medium used was Postgate's medium with lactate as the electron donor and carbon source. The medium was supplemented with 2.5% (wt/vol) NaCl and the cultures were grown anaerobically at room temperature. Weight loss values were determined to evaluate whether corrosion had occurred after a two year exposure. Samples were also examined using ESEM after one year and after 21 months of exposure to determine the presence of microorganisms and/or the formation of a biofilm.

RESULTS AND DISCUSSION

Dimethylsilcones and the Mechanical Sensing Model

Abiotic Electrochemical Measurements - In initial tests with the dip coated samples, as discussed above, corrosion occurred at the edges of these sample due to thinning of the coatings and bare areas on sharp edges. Defects were readily detected when the samples were immersed in the chloride solution and polarized anodically (i.e. current densities increased as the potential was made more positive). See for example the curve in Figure 1 labeled "PEG Coated Steel Defect in Coating". Also, corrosion was visibly evident at the edges of the sample. These results are important in that they show that the polarization technique could be used to quickly determine the presence of pores or defects.

Figure 1 also shows anodic polarization curves for two 4340 steel samples and two PEG coated steel samples without defects. Anodic polarization curves show that the current density of untreated 4340 steel samples increased as the potential increased (made more positive) indicating general corrosion. However, current densities on 4340 samples with defect-free PEG-015 coatings (produced by dipping) remained very low (less than 0.6 nA/cm^2). Subsequent, examination of the defect free silicone coated 4340 steel surfaces using optical and scanning electron microscopy showed no sign of general or localized corrosion. In contrast, untreated 4340 steel experienced uniform corrosion with some pitting. Visible corrosion was noted on untreated 4340 samples after less than 1 hour of exposure to the chloride solution. Corrosion rates for untreated 4340 steel and nominal corrosion rates for defect-free PEG coated steels are listed in Table 1. Measured current values for PEG coated samples were at the lower detection limit of the potentiostat so that calculated current densities and corrosion rates presented in Table 1 are nominal values. The important point is that defect free PEG coatings were produced in thicknesses of engineering significance and these coatings provided an effective barrier layer that prevented the underlying metal from corroding.

Figure 2 shows polarization resistance values obtained from 4340 steel and 4340 steel coated with PEG-060 immersed in quiescent, 0.6M NaCl solutions for 10 days. These values were determined using *ac* impedance. Impedance results and visual examinations show that the 4340 steel undergoes corrosion shortly after immersion. Polarization resistance values for the silicone coated 4340 steel remain high (10^8 ohm/cm^2) for the 10 day period indicating that the PEG was a good barrier coating i.e. there were no pinholes in the coating and the protective nature of the PEG did not degrade during exposure. A decreasing value of polarization resistance would indicate that ions or water penetrated the coating or that ions were being transported through the film, and that charge transfer reactions were occurring at the coating/metal interface. In general, a polarization resistance value of 10^6 $ohm\text{-}cm^2$ or below indicates a non-protective coating.

Exposure of PEGs to an Estuary Environment - PEG-015 samples were made in four degrees of rigidity by use of different ratios of pre-polymer to cross-linking catalyst. The silicones were then immersed in the estuary at the Smithsonian Laboratory in Edgewater, Maryland for six weeks. The intent of this work was to determine if macroorganisms such as barnacles would attach to the PEG surfaces. No barnacle settlement was observed on the silicone samples but barnacles did settle on the polystyrene rack used to hold the samples during immersion. There was some algal growth starting at the edge of the samples where the silicone samples were in contact with the sample holder. This growth progressed toward the center of the silicone samples. No sample was completely overgrown and all showed a

"clear" area in the central area of the samples. Microscopic observation of the "clear" area showed a sparse population of the remnants of larval tubes of invertebrates such as amphipods, a few nematodes, swarming organisms similar to paramecia, and a few strands of algal forms traversing the surface. All could be easily dislodged from the surface with a dissecting needle, unlike the barnacles, tubeworms, or bryozoans that were firmly attached on the hard surfaces of the test rack. The most significant finding of this work was that when samples were arranged in the order of apparent overgrowth, the order was that more growth was observed on the more cross-linked samples, the least growth on the least cross-linked samples, with the exception of one highly cross-linked surface which remained relatively "clean". This ordering would be predicted by a mechanical sensing mechanism of microbial attachment.

Results of exposure to the estuary waters showed that the degree of colonization by organisms was less on the silicone surfaces than on the rigid surfaces. As mentioned above, a property of silicones which may be pertinent in preventing or inhibiting colonization by microorganisms is the high chain mobility [22-25]. If mobility is very high, there may not be perceptible stresses induced in an attaching organism so that stress-induced change in the membrane ion flux ratio does not occur. In short, the organisms do not know that they are near a solid surface and therefore do not undergo the metamorphical changes necessary for attachment. Another possibility for the resistance to colonization of these materials is that their interaction with the surface of potential colonizing organism surfaces is weak because no strong bonding can occur. This latter idea is supported by the observations in the medical community that stainless steel heart valves polished with stearic-acid resist "fouling" in the blood stream. Baier[25] observed that the polishing process coats the metal with a layer of the long-chain fatty acid, so that the surface presented to the medium is a close-packed layer of terminal methyl groups. This surface was suggested to be responsible for the non-fouling nature of the surface because the clean stainless steel became "fouled" within a short time after implantation. Dimethylsilicone chains also present a surface rich in methyl groups to the environment, so that there is an element of support for this mechanism, especially since the close-packed chains of long-chain fatty acids are not generally considered to be mobile. As other materials bearing methyl-terminated alkyl chains have not proven to be as non-fouling as DMS, it is possible that both factors are involved in the non-fouling phenomenon.

Metals

The metals that were exposed to the media with and without SRB were selected to determine if there is a relationship between microbial colonization, the type of metallic substrate, and corrosion . In general, tantalum, tungsten, chromium, niobium, and zirconium are neither a major or minor nutrient required by microorganisms; molybdenum is used as trace nutrient, and iron (from 4340 steel) is a major nutrient [26]. Results from the two year exposure show that weight loss varied as to whether the greatest weight loss was observed for the samples exposed to the SRB or the Control (no bacteria added to the Postgate's media). Table 1 lists the weight loss where each value is the average for at least two samples. A ranking as to the weight loss (from highest to lowest) for a given substrate in the various media is given below.

steel	49Z > Control > CG59
tantalum	CG59 > 49Z > Control
chromium	CG59 > 49Z > Control
tungsten	Control > 49Z > CG59
molybdenum	Control > 49Z > CG59
niobium	Control > CG59 > 49Z
zirconium	Control = CG59 = 49Z = 0

As can be seen in Table 1, the weight loss for the 4340 steel was clearly highest for samples exposed to 49Z SRB (191.6 mg), whereas, the weight loss in CG59 SRB (94.6 mg) was lower than the weight loss for the Control (136.2 mg). In prior work, dealloying of chromium was observed in 4340 steel coupons exposed to 49Z and CG59 SRB consortia after a 6 month exposure period[19]. For tantalum and chromium there was no weight loss in the Control media and a very small weight loss for the samples exposed to SRB.

Weight loss for the tungsten samples exposed to Control media alone was the next highest weight loss value observed (29.5 mg); while molybdenum samples exposed to Control media had the third highest weight loss value (15.4 mg). These weight loss values were significantly higher than the values observed for the tungsten and molybdenum exposed to the media with SRB. Weight loss for molybdenum samples exposed to the 49Z and CG59 (6.1 and 2.0 mg, respectively) was greater than the weight loss of the tungsten samples exposed to the 49Z and CG59 (2.4 and 0.7 mg, respectively). In the case of niobium, there was a small weight loss for the Control sample and essentially no weight loss for the samples exposed to SRB. Zirconium had the best results in that no weight loss was observed for samples exposed to any of the media.

ESEM was used to characterize the surfaces at one year and at twenty-one months after exposure to the CG59 and 49Z SRB. The ESEM examination showed that all metal samples were colonized to some extent and that patchy biofilms were present. These biofilms were not noticeable by visual examination of the samples.

The highest weight loss values observed occurred for the 4340 steel samples in all media. The next highest weight loss values observed were for the molybdenum. When the weight loss values for the molybdenum and tungsten samples exposed to SRB were compared to the Control samples, the weight loss for the samples exposed to the SRB were significantly less than those of the Control, i.e. there was an inhibitory effect on dissolution. This inhibitory effect was also observed for the 4340 steel exposed to CG59 consortia as compared to the Control. The observation that some SRB cultures can have an inhibitory effect on metal dissolution has been previously reported[27]. With the exception of tungsten exposed to 49Z (2.4 mg weight loss), the weight loss values for tantalum, chromium, niobium, and zirconium were negligible (below 1.0 mg).

SUMMARY

Two candidate polydimethylsiloxane polymers (PEG-015 and PEG-060) were selected to test a mechanical sensing mechanism of microbial attachment. The steel coated with these materials had essentially non-existent corrosion rates, extremely stable anodic and cathodic polarization behavior, and high polarization resistance values. These results show that the silicone compounds produce an effective barrier to abiotic corrosion in 0.6M NaCl solutions. The most significant finding of this work was that, after exposure to an estuary environment, more colonization was observed on the more cross-linked samples and the least growth was seen on the least cross-linked samples, with the exception of one highly cross-linked surface which remained relatively "clean". This ordering would be predicted by a mechanical sensing mechanism.

Environmental scanning electron microscopy (ESEM) characterization of zirconium, chromium, niobium, tantalum, molybdenum, tungsten, and 4340 steel to Postgate's media containing sulfate reducing bacteria (SRB) showed that patchy biofilms formed on all samples. The weight loss for the 4340 steel and the molybdenum were significantly higher

than weight loss for other metallic samples. In the case of the zirconium, chromium, niobium, and tantalum samples, weight loss after a two year exposure was negligible.

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Table 1. Abiotic corrosion behavior of 4340 steel with and without silicone treatments exposed to quiescent, 0.6 M NaCl solutions.

Treatment	Exposure (Days)	Coating Thickness (μm)	Corrosion Current Density ($\mu A/cm^2$)	Corrosion Rate (mils/year)
None	1	-	9	4.8
PEG-060	1	860	1×10^{-3}*	4.6×10^{-4}
PEG-060	10	250	1×10^{-3}*	4.6×10^{-4}
PEG-015	1	230	1×10^{-3}*	4.6×10^{-4}
PEG-015	1	47	1×10^{-4}*	4.6×10^{-5}
PEG-015	1	22	1×10^{-4}*	4.6×10^{-5}

*Nominal values - the measured current values are at the lower detection limit of the potentiostat.

Table 2 . The weight loss data for 4340 steel and pure metals after a two year of exposure.

Material	Control	CG-59 (hydrogenase -)	47Z (hydrogenase +)
	grams ($x10^{-3}$)	grams ($x\ 10^{-3}$)	grams ($x10^{-3}$)
4340 steel	136.2	94.6	191.6
chromium	0	0.4	0.3
molybdenum	15.4	2.0	6.1
tungsten	29.5	0.7	2.4
zirconium	0	0	0
niobium	0.5	0.1	0
tantalum	0	0.9	0.4

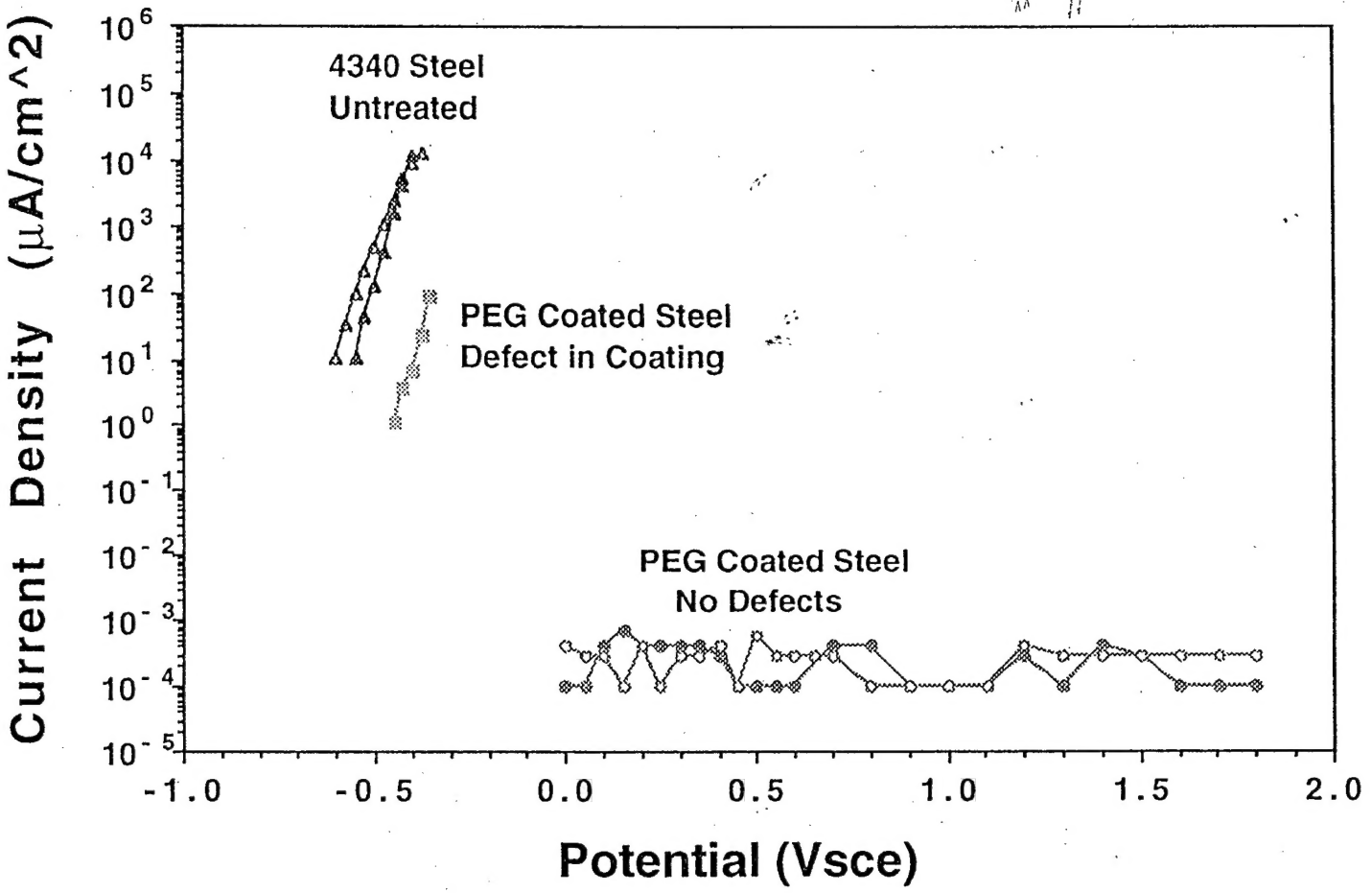

Figure 1. Anodic polarization curves for two 4340 steel samples, a PEG coated steel sample with a defect(s) in the coating, and two PEG coated steel samples without defects.

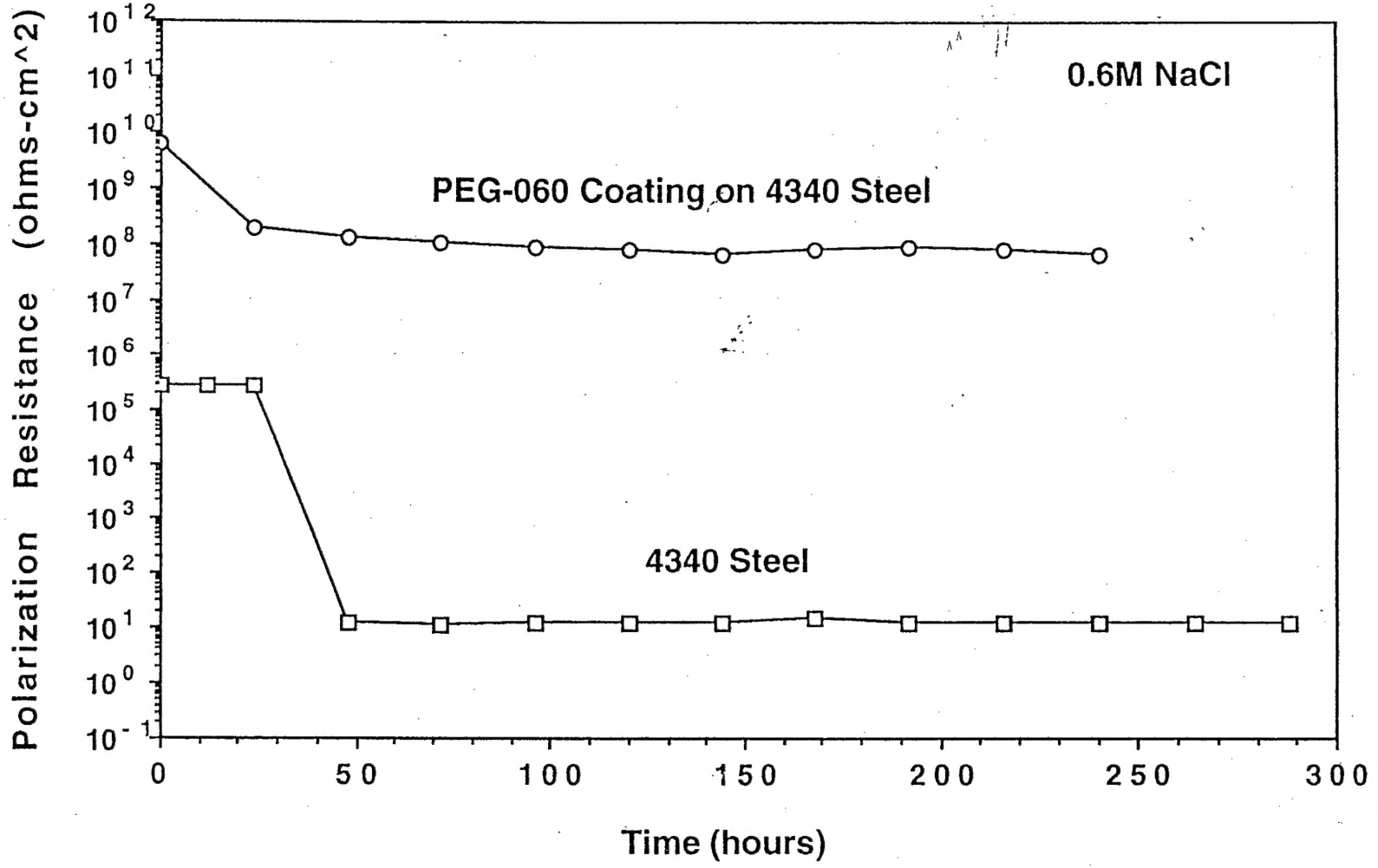

Figure 2. Polarization resistance vs. time for 4340 steel with a PEG-060 coating and untreated 4340 steel.